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Determining the Behavior of Water in Buttermilk Cheese with Polymerized Whey Protein Using Differential Scanning Calorimetry and Nuclear Magnetic Resonance Analysis

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Abstract: In this research, the behavior of water in buttermilk cheese with the addition of polymerized whey proteins was determined. Various parameters of the produced cheese, such as texture, color, water activity, and unbound protein fraction, were examined. Four different samples of buttermilk cheese were prepared, including no addition of whey protein concentrate (BMC); addition of whey protein concentrate (BMC/WPC; 5.62%, *w*/*v*), single-heated polymerized whey protein (BMC/SPWP; 28%, *w*/*v*), and double-heated polymerized whey protein (BMC/DPWP; 28%, *w*/*v*). Differential Scanning Calorimetry (DSC) analysis showed that the highest percentage of freezable water in the water fraction and the lowest of unfreezable water was found in buttermilk cheese with WPC and buttermilk cheese with DPWP. Nuclear magnetic resonance (NMR) analysis showed that the relaxation times were longer in buttermilk cheese with WPC, compared to buttermilk cheese with SPWP and DPWP. Single heat treatment of whey proteins increased stickiness almost 3-fold, and double heat treatment had almost a 2-fold increase in work of shear of cheese samples. The calculated total color difference (ΔE) of the cheese samples suggested that those with polymerized whey protein may increase consumer acceptability.

Keywords: buttermilk cheese; polymerized whey protein; differential scanning calorimetry; nuclear magnetic resonance; texture; color

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1. Introduction

Buttermilk is a by-product of butter production and is used to improve the quality of yogurts [1], formulation of compound milk chocolate [2], and as an encapsulating agent [3] due to its functional and technological properties. The literature has shown that the management of buttermilk most often applies to cheese making [4–7]. The main commercial type of buttermilk is sweet buttermilk. Cultured buttermilk and whey buttermilk are also available, but the ability to make cheese is the most important aspect [8]. The composition of sweet buttermilk is similar to skim milk, however, due to the content of phospholipids, it exhibits emulsification properties, water-retaining capacity, and lower foaming capacity than skimmed milk [9]. The composition of buttermilk contains all the water-soluble ingredients as cream, including milk protein, lactose, minerals, and milk fat globule membrane (MFGM). The presence of MFGM in buttermilk provides a greater amount of phospholipids compared to milk [10], because the shredded membranes of milk fat globules that have been interrupted in the process of whipping the cream mostly migrate to the water phase [11].

The uncomplicated processing of buttermilk to obtaining unripened buttermilk cheese can be easily implemented in the dairy industry [6]. In traditional cheese-making, casein is almost the exclusive curd structure, and almost all of the β-lactoglobulin and α-lactalbumin fraction are lost in the whey. Therefore, the increase of the pro-health value and technological quality of buttermilk cheese can be achieved via the introduction of whey protein concentrates (WPC) into buttermilk [12]. According to reports, the utilization of WPC in cheese-making improves the rheological and sensory properties of the processed cheese [13], which can be used as a substitute in the production of low-fat cheeses [14], alter the concentration of volatile compounds in cheese [15], and obtain edible coatings used for preserving fresh-cut cheese [16]. Furthermore, the addition of whey proteins may contribute to textural changes, as well as changes in the degree of water cross-linking in the produced cheese. Both aspects can influence the sensory characteristics of the product and the economic cost of production [17]. Therefore, it is important to determine the distribution and organization of the aqueous phase within the emulsions of cheese.

The differential scanning calorimetry (DSC) method [18] has been employed to monitor the thermal properties of cheese, whereas nuclear magnetic resonance (NMR) examines the physicochemical changes [19]. Reports have shown that DSC analysis describes the protein and polysaccharide interactions in cheese [20], elaborates the water-protein-fat interactions in pasta filata cheeses [21], or determines the phase transition of various cheese packaging materials [22]. However, NMR analysis focuses on cheese triacylglycerols for the quantitative determination of individual fatty acids and for the classification of cheese samples according to the type of production, origin, and variety [23]. Moreover, Conte et al. [19] suggested that the use of NMR is a promising technique for determining physicochemical changes during cheese maturation, and, according to Gonçalves et al. [24], NMR analysis is a tool to identify mozzarella cheese subjected to various cooling conditions.

The aim of our research was to determine the behavior of water in buttermilk cheese with increased content of whey proteins after the heating process using DSC and NMR analysis. The polymerization process of the whey protein concentrate was carried out using two heating cycles. NMR measurements of organization and distribution of the aqueous phase within the emulsions that constitute the cheese were also analyzed. DSC and NMR analyses are effective tools not only for monitoring physicochemical changes in cheese making, but also for developing standardization of the production protocol. The obtained results may have an impact on the development of dairy products in the sector of products with increased whey protein content, in line with consumer expectations. Therefore, the research gives greater insight into the analysis of texture and color parameters of the produced cheese samples. The introduction of whey protein concentrates after the polymerization process may contribute to the expansion of the range in cheese making with a completely new and/or innovative product.

2. Materials and Methods

2.1. Preparation of Polymerized Whey Protein (PWP)

Preparation of PWP (28%, w/v): whey protein (WP) concentrate powder (WPC80; 79.49% proteins, 6.31% fat, 5.15% lactose, 4.81% water, 1.26% ash) (Spomlek, Radzyń Podlaski, Poland) was dissolved in cold purified water and allowed to stand at 4 °C for 12 h. Solution was heated at 85 °C for 30 min and then quickly cooled to room temperature [25], Thus single-heated polymerized whey protein (SPWP) was obtained. Solution was then reheated under the same conditions described in the first heating process. Heating led to the formation of double-heated polymerized whey protein (DPWP), which was then rapidly cooled to room temperature in ice water under agitation in accordance with the methodology described by Bielska et al. [26].

2.2. Buttermilk Cheese Preparation

Buttermilk which was left over after the industrial production of butter from cream (Great Poland, Poland). The cheese from buttermilk was prepared with addition: whey protein concentrate powder (5.62%, w/v) and whey protein concentrate after the polymerization process (28%, w/v). The buttermilk (20 L) with the whey proteins (in a ratio of 0.23 L of solution PWP for every 1 L of buttermilk) were mixed in double coat cheese kettle type SKM50 (Plevnik, Dobrova, Slovenia) with an automatic processor GPC 145, equipped with automatic propeller stirrer at 15 °C for 10 min, 36 rpm. Four samples of cheese from buttermilk were prepared: no addition of whey protein concentrate (BMC); addition of whey protein concentrate (BMC/WPC); addition of single-heated polymerized whey protein (BMC/SPWP); and addition of double-heated polymerized whey protein (BMC/DPWP). Technical and technological conditions for the production of buttermilk cheese was in accordance with the methodology [12]. The cheeses were stored under refrigerated conditions at 4 ± 0.2 °C and analyzed no later than the next day after production (within the first 24 h).

2.3. Differential Scanning Calorimetry (DSC)

Differential scanning calorimeter DSC 7 (Perkin Elmer, Norwalk) equipped with an Intracooler II and Pyris software was used to investigate the water behavior in cheese samples. DSC was calibrated using indium (m.p. 156.6 °C, ΔH_f = 28.45 J/g) and n-dodecane (m.p. −9.65 °C, ΔH_f = 216.73 J/g). Cheese samples (9–10 mg) were weighed into 20 µL aluminum pans (Perkin Elmer, No. 0219-0062) and hermetically sealed. The reference was an empty, hermetically sealed aluminum pan. Nitrogen (99.999% purity) was used as the purge gas. The sample pan was placed in the calorimeter at 5 °C. The following time—temperature program was set: (1) heating and isotherm for 1 min at 70 °C; (2) cooling at 5 °C/min to −40 °C; and (3) heating at 5 °C/min to 70 °C. The following parameters were analyzed from the second melting curve: the temperatures of ice melting T_{onset}, T_{peak}, and the enthalpy ΔH_m of ice melting, calculated per 1 g of water. The content of freezable water (FW) in the water fraction was calculated as:

$$FW = \left(\frac{\Delta H_m}{\Delta H_{ref}}\right) \times 100 \quad (1)$$

where ΔH_m is the enthalpy of ice melting per unit mass of water contained in cheese (J/g), ΔH_{ref} is the enthalpy of ice melting for samples of pure water, equal to 333.7 J/g.

The unfreezable water content (UFW) was calculated as:

$$UFW = 100 - FW\ (\%) \quad (2)$$

where FW is freezable water.

2.4. Low-Field Nuclear Magnetic Resonance (NMR)

The samples were cut from cheese in form of a cylinder: 0.8 cm in diameter and 0.6 cm in height. Measurements of the spin-lattice (T_1) and spin-spin (T_2) relaxation times were performed using a pulse NMR spectrometer operating at 20 MHz (Ellab, Poland). The inversion-recovery (π−TI−$\pi/2$) impulse sequence [27] was applied for measuring T_1 relaxation times. Distances between impulses (TI) were changed within the range of 1 to 200 ms and the repetition time was from 15 s. In each scan, 32 FID signals and 110 points from each FID signal were collected. Calculations of the spin-lattice relaxation time values were performed with the assistance of the CracSpin program [28]. This program is for calculating relaxation parameters from experimental data using spin grouping approach. Marquardt's method of minimization was applied for fitting multiexponential decays. The accuracy of the relaxation parameters was estimated with a standard deviation. Time changes of the current value of FID signal amplitude in the employed frequency of impulses were described using the following formula [29]:

$$M_z(TI) = M_0 \times \left(1 - 2e^{\frac{-TI}{T_1}}\right) \quad (3)$$

where $M_z(TI)$ is the actual magnetization value, M_0 is the equilibrium magnetization value, TI is the distance between impulses, and T_1 is the time of relaxation.

A monoexponential magnetization recovery was found, which meant that the system relaxed with one T_1 spin-lattice relaxation time. T_2 spin-spin relaxation times measurements were recorded using the pulse train of the Carr-Purcell-Meiboom-Gill spin echoes $(\pi/2 - TE/2 - (\pi)n)$ [27]. The distance between π (TE) impulses amounted to 1 ms. The repetition time was 15 s. The number of spin echoes (n) amounted to 100. Ten accumulation signals were employed. To calculate the spin-spin relaxation time values, the authors applied the adjustment of values of the echo amplitudes to the formula [30]:

$$M_{x,y}(TE) = M_0 \sum_{i=1}^{n} p_1 e^{\frac{-TE}{T_{2i}}} \tag{4}$$

where $M_{x,y}$ (TE) is the echo amplitude; M_0 is the equilibrium amplitude; TE is the distance between π impulses; and p_i is the fraction of protons relaxing with T_{2i} spin-spin time.

The calculations were in accordance with the methodology Tomaszewska-Gras et al. [18].

2.5. Texture Profile Analyses

The measurement of selected cheese texture parameters was performed using a texturometer (Stable Micro Systems Ltd., Surrey, UK) equipped with an HDP/SR attachment. A set of male and female acrylic cones with 90° angles were used. Samples were filled into the female (lower) cone with a spatula and pressed lightly to eliminate air pockets. Test Speed 3.0 mm·s^{-1}, Post–Test Speed 10.0 mm·s^{-1}, distance 23.0 mm, samples of 8 g). Results were recorded using Texture Exponent E32 version 4.0.9.0 software (Godalming, Surrey, UK).

2.6. Total Color Difference

Instrumental color measurements were based on the CIELAB color space. Measurements were performed with a geometry SPIN using an X-Rite SP-60 camera (X-Rite, Grandville, MI, USA) according to Bielska et al. [26]. Total color difference (ΔE) was calculated compared to BM cheese without the addition of whey proteins using the following equation:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \tag{5}$$

where L is lightness; a is redness to greenness; b is yellowness to blueness.

The calculations assumed that ΔL = 88.89, Δa = −0.23, and Δb = 12.93.

2.7. Water Activity

The water activity was measured with an AquaLab Series 4TE instrument (Decagon Devices Inc., Pullman, WA, USA). Samples of v = 15 mL and temperature at 15 °C were placed in a DE 501 measurement vessel (Decagon Devices Inc., Pullman, WA, USA).

2.8. Unbound Protein Fraction

Unbound protein fraction (UPF, %) was accomplished using a centrifugation method. Whey (25 cm^3) was centrifuged (model 260; MPW MED Instruments, Warsaw, Poland) under relative centrifugal force (RCF) = 10 732 g, rotor angle 30° (RPM 10 062 g) at 4 °C per 15 min. Precipitate contained more than 98% of proteins in total solids. The precipitate was collected and weighed, and the unbound protein fraction was calculated according to the following equation:

$$UPF\ (\%) = (m_1 100)/m_2 \tag{6}$$

where m_1 is the weight in grams of the precipitate after centrifugation and m_2 is the weight of whey in grams.

2.9. Statistical Evaluation

The samples were evaluated by a two-way analysis of variance followed by Tukey's HSD post hoc test. Verification of statistical hypotheses was achieved using a level of significance of α = 0.05. Data were analyzed using Statistica data analysis software, version 13 (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results and Discussion

3.1. Differential Scanning Calorimetry

DSC enables quantitative and qualitative determination of physicochemical changes in heat flow in a sample during phase transition, such as melting [31]. Deynichenko et al. [32] report that stabilizers and gelling agents affect the water condition in food products. In Figure 1, an example of an ice melting curve of BMC sample is shown with indication of measured parameters of onset temperature (T_{onset}) and peak temperature (T_{peak}). Table 1 shows the results of DSC analysis of ice melting phase transition in buttermilk cheese in order to examine the behavior of water, which was measured after crystallization under controlled conditions with a scanning rate of 5 °C/min. During the cooling process (results not shown), peaks related to the liquid-solid exothermic phase transition were observed. At approx. −15 °C, the first-order transition occurred, corresponding to the crystallization of water, associated with a sharp and narrow peak. Using the cooling and heating procedure, it was possible to calculate from the melting curve the content of freezable water (FW), which was related to free water, as well as unfreezable water (UFW), namely bound water.

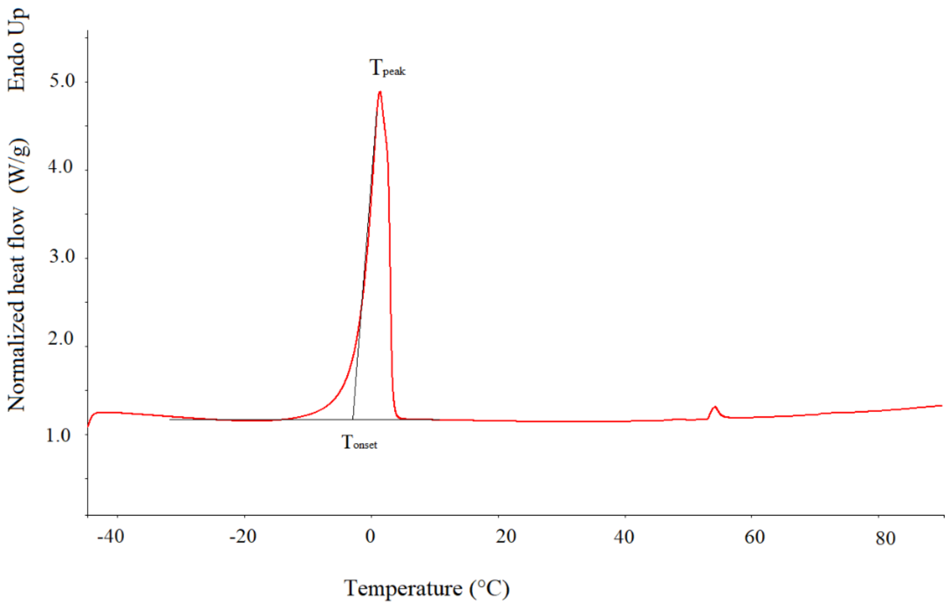

Figure 1. DSC melting curve of BMC cheese sample showing ice melting peak. T_{onset}: onset temperature; T_{peak}: temperature at maximum heat flow value.

It was determined that the percentage of FW in cheese samples ranged from 74.4% to 77.91%. The highest percentage of FW and lowest UFW was found in BMC/WPC and BMC/DPWP samples, where the remaining samples showed significant differences (BMC, BMC/SPWP) ($p \leq 0.05$). Significant differences were also observed in the mean values of peak temperatures (T_{peak}) and onset temperatures (T_{onset}), as well as ice melting enthalpies (ΔH_m). The lowest peak temperature was determined for BMC/SPWP sample (0.98 °C) and the highest for BMC/DPWP cheese sample (1.77 °C). The DSC results showed that the single heating process of WPC solution used for the production of cheese is necessary and optimal for the binding of water in cheese, since in the sample without heating (BMC/WPC), the amount of free water (FW) was higher, similarly as in the case of sample with a double heating of WPC solution (BMC/DPWP). Feng et al. [33] reported that cottage cheese contains small globules of fat that are protected by protein layers, which may justify its higher melting point. According to Brożek and Bohdziewicz [31], the DSC method is an effective tool for determining and comparing the thermal properties of cottage cheese with different fat content. However, according to Sun et al. [34], it is also possible to determine the thermal properties of whey protein concentrates and their modifications (caused by the heating process and/or the addition of soy lecithin) as a result of changes to the surface hydrophobicity of whey proteins. Thus, not only the ingredients of buttermilk (phospholipids or MFGM particles), but also the conditions of further processing can promote shaping rennet gel properties and model cheese manufacturing. Morin et al. [35] showed that milk with raw cream buttermilk started to coagulate significantly faster than with buttermilk from pasteurized cream. This was not a result of pasteurization, but the ability of the rennet to hydrolyze. The deposition of denatured whey proteins and MFGM proteins on casein micelles may slow hydrolysis [35].

Table 1. DSC parameters of buttermilk cheese with polymerized whey protein.

Parameters Ice Melting	**Buttermilk Cheese**			
	BMC	**BMC/WPC**	**BMC/SPWP**	**BMC/DPWP**
Temperature				
T_{onset} (°C)	-2.09 ± 0.00 ^{a}	-2.25 ± 0.01 ^{ab}	-2.43 ± 0.01 ^{b}	-2.07 ± 0.21 ^{a}
T_{peak} (°C)	1.44 ± 0.09 ^{ab}	1.24 ± 0.06 ^{ab}	0.98 ± 0.05 ^{a}	1.77 ± 0.43 ^{b}
Enthalpy ΔH_m (J/g of sample)	193.32 ± 0.49 ^{a}	203.35 ± 0.83 ^{b}	194.40 ± 2.22 ^{a}	203.10 ± 3.12 ^{b}
Enthalpy ΔH_{ice} (J/g of water)	247.28 ± 0.63 ^{a}	258.94 ± 1.06 ^{b}	247.35 ± 2.83 ^{a}	258.37 ± 3.97 ^{b}
Freezable water (g/100 g of water)	74.40 ± 0.19 ^{a}	77.91 ± 0.32 ^{b}	74.43 ± 0.85 ^{a}	77.74 ± 1.19 ^{b}
Unfreezable water (g/100 g of water)	25.60 ± 0.19 ^{b}	22.09 ± 0.32 ^{a}	25.57 ± 0.85 ^{b}	22.26 ± 1.19 ^{a}

BMC: buttermilk cheese; BMC/WPC: buttermilk cheese with whey protein concentrate; BMC/SPWP: buttermilk cheese with single-heated polymerized whey protein; BMC/DPWP: buttermilk cheese with double-heated polymerized whey protein; a,b: different small letters in superscript in the rows indicate between parameters statistically significant differences (p = 0.05).

3.2. Low-Field Nuclear Magnetic Resonance

Molecular dynamics analysis of the buttermilk cheese samples with the addition of whey proteins before and after heating is presented in Table 2. Tracking of changes in the molecular dynamics of protons was used to evaluate butter cheeses and their stability. Measurements of spin-lattice T_1 and spin-spin (short T_{21}, long T_{22}) relaxation times were conducted using a pulse NMR spectrometer that reflected the relaxation processes of water molecules in the cheese samples. The spin-spin relaxation times described two components of relaxation times. Hence, the movement of protons related to bound water and bulk fractions was observed according to Cameron and Fullerton [36]. At the same time, registering only one spin-lattice time value in each sample meant that proton exchange was faster than the lifetime of individual fractions. The observed relaxation times were significantly longer in BMC/WPC cheese, and shorter in BMC/SPWP and BMC/DPWP cheese compared to the control cheese. In addition, comparable T_{21} values were recorded, hence, similar interaction dynamics occurred between water molecules and proteins. In cheese with BMC/DPWP, a decrease in the value of spin-net relaxation times was observed with a simultaneous increase in the value of the long component of spin-spin relaxation times (T_{22}). Therefore, the amount of bulk water in relation to bound water decreased over time. However, the mobility of the molecules in the bulk water fraction was greater than that in the control cheese. Presumably, the fat solidified in the cheeses, which resulted in the formation of a gel-like lamellar phase that encases water molecules, created an ordered structure. Spin-spin relaxation occurs within a short time, which meant the arrangement of the water structure between the fatty acid chains. Spin-lattice relaxation describes the interaction of spins with the environment. The protons of water contained in the cheese structure network were mainly responsible for the spin-lattice relaxation times. The fat bound to the protein matrix at sorption sites promoted water removal. At the time of polymer network formation, water molecules were temporarily locked in the nodes of the network. The increase in relaxation times after this stage promoted the removal of water molecules from the knots. The systems related to buttermilk cheeses with whey proteins were characterized by changes in molecular dynamics that arose due to changes in the relaxation times values of the short components. This stemmed from the formation of the structural body of the fat fraction. Under the influence of a small amount of whey proteins, the fatty chains solidified in α-crystal configuration. Over time they formed a gel structure with the participation of water. Therefore, it was important to examine the texture of the cheeses, especially their firmness and adhesion. Similar observations were made by Kruk et al. [37], who suggested that differences in the behavior of water, and thus the macroscopic properties of cheese, result from variations in the relative populations of these fractions and the portion of water molecules bound to proteins. NMR analysis is an effective method to identify the different production chains for the purpose of cheese quality control practice [38,39]. Song et al. [40] showed that the interaction of water with the main macromolecules that create the cheese matrix has a significant effect on the proton relaxation signal. They proved the relationship between casein surface hydrophobicity and protein hydration, which in turn translated into the cheese microstructure. The changes in protein structure in cheese due to proteolysis were associated with the decrease in T_{21} and T_{22}.

Table 2. Molecular dynamics and water activity of buttermilk cheeses with polymerized whey protein.

Parameters	Sample			
	BMC	**BMC/WPC**	**BMC/SPWP**	**BMC/DPWP**
T_1	248.26 ± 16.58 ^{a}	303.46 ± 1.11 ^{b}	244.71 ± 9.21 ^{a}	257.67 ± 1.53 ^{a}
T_{21}	26.61 ± 0.46 ^{b}	32.99 ± 0.44 ^{c}	25.71 ± 0.44 ^{ab}	24.88 ± 1.04 ^{a}
T_{22}	59.17 ± 0.63 ^{a}	72.45 ± 1.25 ^{c}	58.91 ± 0.71 ^{a}	67.03 ± 0.71 ^{b}
p_{21}	32 ± 1 ^{a}	33 ± 2 ^{a}	30 ± 1 ^{a}	31 ± 2 ^{a}
p_{22}	68 ± 2 ^{a}	67 ± 3 ^{a}	70 ± 3 ^{a}	69 ± 1 ^{a}

T_1: spin-lattice relaxation times; T_2: spin-spin relaxation times (short T_{21}, long T_{22}); p_{21}, p_{22}: reflect fractions of protons relaxing with T_{21} and T_{22} times; BMC, BMC/WPC, BMC/SPWP, BMP/DPWP: the same at Table 1; a–c: different small letters in superscript in the rows indicate between parameters statistically significant differences (p = 0.05).

3.3. Texture and Total Color Difference

Giha et al. [41] reported that the general concept of the rheological properties of cheeses mainly refers to the maintenance of their integrated structure, which may impact on the chew length and overall cheese acceptability. Determination of the texture profile of the cheese allows for the characterization of a number of mechanical properties, such as hardness, cohesiveness, adhesiveness, and chewiness [42]. Instrumental analysis of the texture of dairy products is utilized as quality control for the technological process carried out [43]. In this research, it was shown that the introduction of whey proteins to buttermilk during the production of buttermilk cheese caused an almost 4-fold increase in firmness (BMC/WPC, $p < 0.05$) (Table 3, Figure 2). Similar observations are made by Lobato-Calleros et al. [44] who reported that the addition of WPC to the cheese matrix results in a higher protein/fat ratio, and the main factor determining the texture properties of the cheese is the structure of the protein matrix. Presumably higher firmness in WPC buttermilk cheese compared to the control sample was associated with the lower proportion of bound water shown in DSC measurements.

Further modification of the functional properties of whey proteins (single and double heating) did not alter the firmness of the buttermilk cheese ($p > 0.05$), a similar relationship was demonstrated when analyzing the work of adhesion ($p > 0.05$). Analysis work of shear and stickiness of BMC/WPC cheese showed no differences compared to the BMC sample. The texture parameters values did not show a correlation with FW and UFW measured using DSC in cheeses with differently heated PWP. Therefore, in order to correlate the share of free and bound water, each of the texture parameters must be considered separately.

The obtained DSC and texture results showed distinct properties of the cheese samples, which related directly to their future use and even storage capabilities. However, single heat treatment of the whey proteins increased the stickiness by almost 3-fold (BMC/SPWP, $p < 0.05$), and double heat treatment by almost 2-fold the work of shear (BMC/DWPW, $p < 0.05$) compared to BMC/WPC sample. In the literature, the introduction of WPC to cheese production is commonly related to the change in the volatile compound profile of cheese [45] and the improvement of texture [46]. The texture of dairy products is also influenced by the whey protein polymerization process [25]. According to Sołowiej et al. [47], polymerized whey protein can be a good replacement for emulsifying salts in cheese production, where they are able to thicken the cheese mass due to good water absorption. In addition, the replacement of PWP emulsifier salts improves the spreadability of processed cheese. Masotti et al. [8] in their review confirm that whey-base can be used to increase cheese yield and that denatured whey protein is preferred to assure their entrapment in the cheese network. Topcu et al. [48] suggest that the factors influencing the texture of cheeses are: protein hydrolysis, reduction of free water, and solidification of milk fat.

Table 3. Texture of buttermilk cheeses with polymerized whey protein.

Parameters	Sample			
	BMC	BMC/WPC	BMC/SPWP	BMC/DPWP
Firmness (g)	5469.50 ± 423.05 ^{a}	20,083.36 ± 2928.53 ^{b}	15,774.46 ± 83.99 ^{b}	25,330.61 ± 4064.40 ^{b}
Work of Shear (g·s)	5593.25 ± 601.48 ^{a}	14,130.35 ± 1357.68 ^{a}	16,079.89 ± 954.08 ^{ab}	26,885.77 ± 5078.48 ^{b}
Stickiness (g)	474.07 ± 20.91 ^{a}	435.86 ± 298.90 ^{a}	1258.43 ± 7.27 ^{b}	1582.00 ± 23.84 ^{b}
Work of Adhesion (g·s)	30.62 ± 13.21 ^{a}	155.54 ± 44.51 ^{b}	105.65 ± 3.73 ^{ab}	115.12 ± 7.50 ^{ab}

BMC, BMC/WPC, BMC/SPWP, BMP/DPWP: the same at Table 1; a,b: different small letters in superscript in the rows indicate between parameters statistically significant differences ($p = 0.05$).

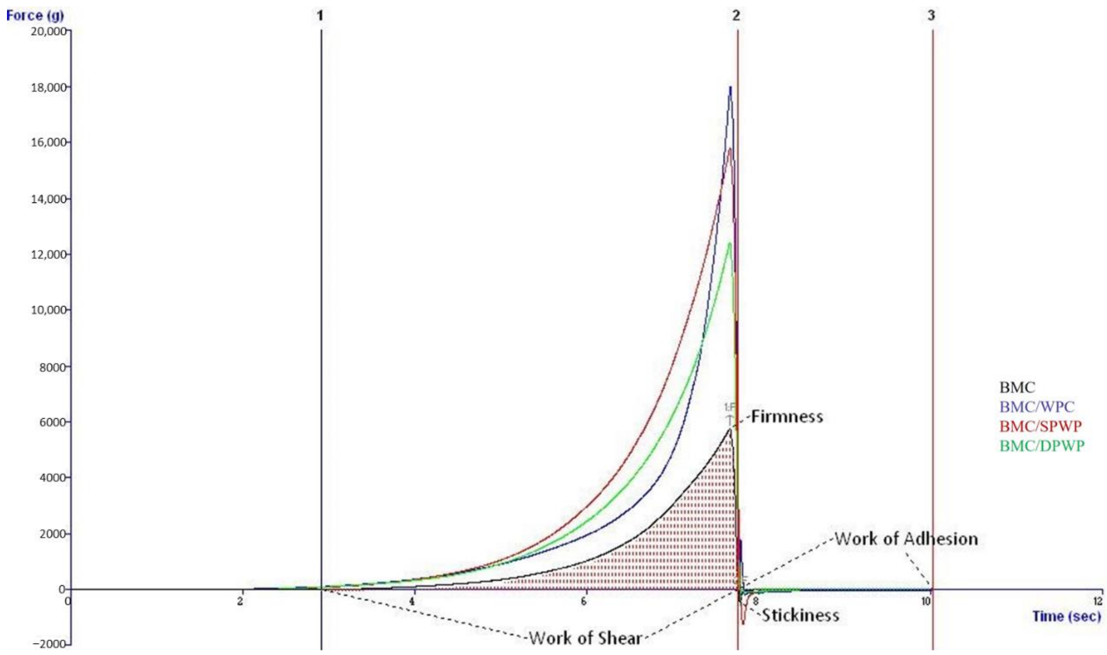

Figure 2. Texture of buttermilk cheeses with polymerized whey protein. BMC, BMC/WPC, BMC/SPWP, BMP/DPWP: the same at Table 1.

Color changes can be measured as the distance vector between the initial color values and the actual color coordinates, and the total color difference can be obtained (ΔE) [49]. In our research, total color difference analysis showed that the cheese with addition of whey proteins (BMC/WPC) was darker than the other samples. Calculated ΔE for BMC/WPC, BMC/SPWP, and BMC/DPWP cheese samples were 9.33, 1.72, and 2.91, respectively (Figure 3). As suggested by Vichi et al. [50] and Liu et al. [51], if ΔE is above the value of 3.3, color is considered unacceptable. Texture and color may correlate with consumer ratings of overall desirability [52]. D'Incecco et al. [53] report that ΔE values higher than 3 are in the threshold for a clearly discernible color difference.

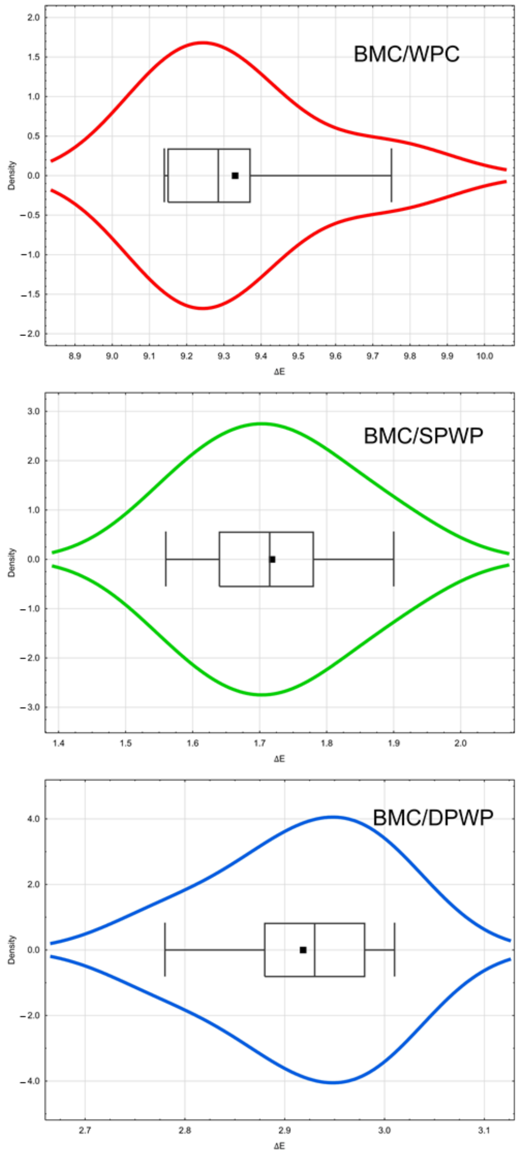

Figure 3. Total color difference of buttermilk cheeses with and without addition of whey protein. BMC/WPC, BMC/SPWP, BMP/DPWP: the same at Table 1; ΔE, total color difference.

The use of polymerized whey proteins in cheese production leads to innovative of dairy product, while simultaneously having a chance to be sensory accepted. This is indicated by the degree of water binding, and thus no leakage of whey from the product and an increased proportion of proteins. In addition, texture parameters indicate the correctness of slicing cheese and biting in the mouth. Therefore, in the future, it is advisable to conduct a sensory profile assessment with the use of descriptors and consumer tests.

3.4. Water Activity and Unbound Protein Fraction

The heating process did not cause any significant changes ($p > 0.05$) in the water activity analysis of the buttermilk cheese samples (Table 4). However, the process of heating whey proteins had a significant impact on the degree of binding of the protein fraction in cheese ($p < 0.05$). The whey after the production of BMC/WPC buttermilk cheese showed 8-fold more precipitate than BMC/SPWP sample ($p < 0.05$). From a technological point of view, the smaller the sludge obtained after centrifugation of the whey after the production of buttermilk cheese, the more proteins remained in the cheese. Therefore, it is worth extending the research with a detailed economic analysis of the use of the raw material (e.g., cheese yield). Moreover, according to Szkolnicka et al. [7], the yield of buttermilk cheese production may result from the ability of MFGM components, including phospholipids, to retain and absorb whey in the cheese curd.

Table 4. Water activity and unbound protein fraction cheeses with polymerized whey protein.

Parameters	Sample			
	BMC	BMC/WPC	BMC/SPWP	BMC/DPWP
Water activity	0.9711 ± 0.0013^{b}	0.9666 ± 0.0001^{a}	0.9687 ± 0.0024^{ab}	0.9676 ± 0.0002^{ab}
UPF (%)	1.26 ± 0.13^{a}	15.00 ± 1.91^{b}	1.86 ± 1.22^{a}	5.40 ± 0.13^{a}

UPF: unbound protein fraction; BMC, BMC/WPC, BMC/SPWP, BMP/DPWP: the same at Table 1; a,b: different small letters in superscript in the rows indicate between parameters statistically significant differences ($p = 0.05$).

4. Conclusions

Polymerization of whey proteins may be a differentiation strategy for buttermilk cheese. The addition of polymerized whey proteins induces water-binding changes in the product, which provide buttermilk cheeses with a textural identity. DSC and NMR analysis were suitable tools for the characterization of the quality of buttermilk cheese. Notably, this is the first study that has evaluated the effect of polymerized whey proteins on soft, unripened cheeses. The success of these studies is that NMR and DSC techniques are useful for characterizing the properties of typical or popular and innovative dairy products. Knowledge about the properties of new products can be used during their application to the dairy plant.

It should also be noted that buttermilk cheese with polymerized whey proteins is a product that follows the trend of rational management of all milk components. Post-production products, such as buttermilk after butter production and whey protein from whey after cheese production, have been combined and developed into a single product. This is of great importance to the minimization of production costs and food waste.

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